Arterial 5-Hydroxytryptamine Transporter Function Is Impaired in Deoxycorticosterone Acetate and \(\text{N}\omega\text{-Nitro-L}-\text{Arginine But Not Spontaneously Hypertensive Rats}

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Abstract—We reported upregulation of the 5-hydroxytryptamine (HT) transporter (5-HTT) protein in peripheral arteries from deoxycorticosterone acetate (DOCA)-salt hypertensive rats. We hypothesized that upregulated 5-HTT may be generally elevated in hypertensive models and, as a consequence, a higher basal concentration of 5-HT, the 5-HT metabolite 5-hydroxyindoleacetic acid, and an increased 5-HT uptake would occur in peripheral arteries of hypertensive rats compared with normotensive rats. We examined 3 hypertension models: DOCA-salt rats, \(\text{N}\omega\text{-nitro-L}-\text{arginine (LNNA) rats, and spontaneously hypertensive rats (SHRs) in our study (systolic blood pressure [mm Hg]): DOCA (D)=197±6, SHAM (D)=112±4, LNNA (L)=228±9, SHAM (L)=128±2, SHR}=172±7, and Wistar-Kyoto [WKY] =121±3). High-pressure liquid chromatography measurements showed lower basal 5-HT concentrations in aorta from DOCA-salt and LNNA rats compared with their SHAM rats but not in SHR compared with WKY. In all of the 5-HT-uptake studies, we used arteries isolated from rats treated with the monoamine oxidase-A inhibitor pargyline to minimize 5-HT metabolism. Exogenous 5-HT was taken up by aorta, and this was inhibited by the 5-HTT inhibitor fluoxetine (1 mol/L) or fluvoxamine (1 mol/L). Total 5-HT uptake and 5-HTT–dependent active 5-HT uptake were decreased in aorta from DOCA-salt and LNNA rats compared with SHAM rats, but this was not observed in SHRs compared with WKYS. Western analysis revealed similar expression of 5-HTT in aorta from WKYs and SHRs as opposed to an upregulated 5-HTT in aorta from DOCA-salt and LNNA-hypertensive rats. Our study suggested that an altered serotonergic system by impaired 5-HTT function might play a role in blood pressure regulation in DOCA-salt and LNNA-hypertensive rats. (Hypertension. 2006;48:134-140.)

Key Word: rats

5-Hydroxytryptamine (5-HT, serotonin) has been recognized as a neurotransmitter and vasoconstrictor for many decades. As the primary protein to take up and release 5-HT,1 the functional 5-HT transporter (5-HTT) is localized to the cellular membrane in tissues of many organs. 5-HTT regulates the entire serotonergic system, and 5-HT receptors function via modulation of extracellular fluid 5-HT concentrations. 5-HTT is known as a target of antidepressant drugs, such as fluoxetine (Prozac), fluvoxamine, citalopram, and paroxetine, and the anorexigen (+)-fenfluramine (Pondimin).

In the cardiovascular system, the function of 5-HTT in anorexigen-induced or hypoxia-induced pulmonary arterial hypertrophy and hypertension has been well studied.2,3 Increased pulmonary arterial 5-HTT expression and function is associated with pulmonary hypertension.4,5 Genetic removal or pharmacological blockade of 5-HTT mitigates development of experimentally induced pulmonary hypertension.6,7 However, the function of the serotonergic system in systemic vasculature is not clear, as it is in pulmonary circulation. Only a few studies have investigated the change of 5-HTT function with hypertension. As a 5-HTT selective inhibitor, fluoxetine was reported to induce acute pressor responses in the rat,8 cause a sustained hypertension during short-term (12-week) fluoxetine treatment in humans,9 and is used in the treatment of severe refractory orthostatic hypotension.10 We reported previously that the functional 5-HTT exists in systemic arterial smooth muscle. Moreover, 5-HTT protein expression was upregulated in aorta from deoxycorticosterone (DOCA)-salt hypertensive rats compared with normotensive rats,11 and fluoxetine and fluvoxamine potentiated 5-HT-induced contraction in aorta from DOCA-salt hypertensive rats but not in aorta from normotensive rats.11 Thus, both expression and contractile function of 5-HTT was altered in DOCA-salt hypertension. We have not, however, measured active uptake of 5-HT into these vessels nor do we know whether the changes that we observed in the DOCA model are indicative of hypertension in general. In this study, we hypo-
esized that the increased expression and function of 5-HTT would be observed in multiple models in hypertension, and, as a result, aortic 5-hydroxyindoleacetic acid (5-HIAA) and 5-HT would increase. To test this hypothesis, we used 2 experimental hypertensive models (the DOCA-salt hypertensive rat and the Nω-nitro-L-arginine [LNNA] hypertensive rat) and a genetic hypertensive model (the spontaneously hypertensive rat [SHR]). We first compared the basal level of 5-HTT concentrations in arteries from hypertensive versus normotensive animals. Second, we investigated 5-HT uptake and 5-HTT expression in arteries from hypertensive versus normotensive animals. Collectively, these studies provide initial insight into the general change of arterial 5-HTT function in hypertension.

Methods

All of the procedures that involved animals were performed in accordance with the institutional guidelines of Michigan State University.

Animal Use

Normal male Sprague–Dawley rats (0.25 to 0.30 kg; Charles River Laboratories, Inc; Harlan Industries, Inc) and male SHR and Wistar-Kyoto rats (WKYs) 12 weeks, Taconic Farms, Inc) were used. In some experiments, animals were injected with vehicle (saline +0.1% ascorbate, IP) or monoamine oxidase A inhibitor pargyline (100 mg/kg, IP) 30 minutes before euthanization with pentobarbital (60 mg/kg, IP).

DOCA-Salt Hypertension

Male Sprague–Dawley rats (0.25 to 0.30 kg; Charles River Laboratories, Inc) underwent uninephrectomy and implantation of DOCA (200 mg/kg) under isoflurane anesthesia as described previously.12 After surgery, DOCA rats were placed on high salt water (1.0% NaCl and 0.2% KCl). On exactly day 14 of treatment, water mixed with LNNA (0.5 g/L). On exactly day 14 of treatment, animals remained on the regimen for 4 weeks.

LNNA Hypertension

Male Sprague–Dawley rats (250 to 300 g; Harlan) were given tap water mixed with LNNA (0.5 g/L). On exactly day 14 of treatment, rat systolic blood pressure (SBP) was measured before euthanization.

Blood Pressure Measurement

SBPs were measured using standard tail cuff methods (pneumatic transducer, Narco).

5-HT Basal Level Measurement

Aorta from normotensive and hypertensive animals were dissected, cleaned, and placed in 75 μL of 0.05 mmol/L sodium phosphate and 0.03 mmol/L citric acid buffer (pH 2.5) containing 15% methanol. Samples were frozen at −80°C for ≥4 hours. Samples were thawed, sonicated for 3 seconds, and centrifuged for 30 seconds (10 000 g). Supernatant was collected and transferred to new tubes. Tissue pellets were dissolved in 1.0 mol/L NaOH and assayed for protein. Concentrations of 5-HIAA and 5-HT in tissue supernatants were determined by isocratic high-pressure liquid chromatography coupled with electrochemical detection. The lower limit of sensitivity for detection of 5-HIAA and 5-HT was 2 to 5 pg per sample.

5-HT Uptake Assay

At room temperature, dissected and washed aorta from pargyline-treated animals were placed in physiological salt solution (mmol/L: NaCl, 130.00; KCl, 4.70; KH2PO4, 1.18; MgSO4·7H2O, 1.17; CaCl2·2H2O, 1.60; NaHCO3, 14.90; dextrose, 5.50; and CaNa2EDTA, 0.03 [pH 7.2]) in 1.5-mL plastic centrifuge tubes containing either vehicle or inhibitor (fluvoxamine, 1 μmol/L or fluvoxamine, 1 μmol/L) for 30 minutes. 5-HT (1 μmol/L) or vehicle (water) was then added for 15 minutes. Experiments were performed at room temperature. Tissues were then briefly dipped in drug-free physiological salt solution and stored in 75-μL tissue buffer at −80°C. 5-HT and 5-HIAA concentration measurements were as described above.

Western Blotting Analysis

Aorta from hypertensive and normotensive animals were dissected directly from animals, cleaned, pulverized in liquid nitrogen, and solubilized in lysis buffer (0.5 mol/L Tris HCl [pH 6.8], 10% sodium dodecyl sulfate, and 10% glycerol) with protease inhibitors (0.5 mmol/L PMSF, 10 μg/mL aprotinin, and 10 μg/mL leupeptin). Homogenates were centrifuged (11 000g for 10 minutes, 4°C) and supernatant total protein measured. Equivalent amounts of arterial protein from tissue samples were separated on 10% SDS polyacrylamide gels and transferred to Immobilon-P membrane for Western analyses using an antibody against the human 5-HTT (H-115, Santa Cruz Biotechnology). Smooth muscle α-actin (1:5000; Oncogene) was used as a marker to ensure equal loading of protein.

Materials

Materials included DOCA, fluoxetine hydrochloride, fluvoxamine maleate, 5-HR hydrochloride, and pargyline hydrochloride (Sigma Chemical Co).

Data Analyses

Data are presented as mean±SEM for the number of animals stated. 5-HIAA and 5-HT content of each sample were quantified using standards run the same day and reported as a concentration relative to protein content (nanogram per milligram of protein). When comparing 2 groups, the appropriate Student t test was used. A P≤0.05 was considered statistically significant.

Results

Basal Level of 5-HT Was Decreased in Aorta From DOCA-Salt Rats and LNNA Rats But Not in SHRs

Our first experiment compared the basal level of 5-HT and its monoamine oxidase A–oxidized metabolite 5-HIAA in aorta from untreated (no pargyline treatment) normotensive and hypertensive rats (Figure 1 A, SBP [mm Hg], DOCA [D] = 197±6, SHAM0 = 112±4, LNNA [L] = 228±9, SHAM0 = 128±2, SHR = 172±7, and WKY = 121±3). Figure 1B demonstrates that 5-HT but not 5-HIAA concentrations were significantly lower in aorta from DOCA-salt compared with SHAM rats (5-HT [mg/mg of protein], SHAM0 = 0.31±0.07 and DOCA = 0.17±0.03; P < 0.05). Both 5-HIAA and 5-HT concentrations in aorta were lower in LNNA rats compared with their control normotensive rats (5-HIAA [mg/mg protein], SHAM0 = 0.73±0.16 and LNNA = 0.10±0.02; P < 0.05; 5-HT [mg/mg protein], SHAM = 0.55±0.16 and LNNA = 0.24±0.03; P < 0.05). No differences were found in aortic 5-HIAA and 5-HT concentrations when comparing values from SHRs to WKYS.

5-HT Uptake Was Reduced in Aorta From DOCA-Salt and LNNA Rats But Not in SHRs

Active 5-HT uptake was measured in aorta from pargyline-treated rats. As reported before,11 5-HIAA concentration was significantly reduced and 5-HT concentration increased in aorta from pargyline-treated rats. By inhibiting 5-HT metabolism, observed changes of 5-HT concentration in aorta after incubation with exogenous 5-HT directly reflect 5-HT uptake in aorta.

Sections of aorta from the same rat were incubated with vehicle, the 5-HTT inhibitor fluoxetine (1 μmol/L), fluvox-
amine (1 μmol/L), 5-HT (1 μmol/L), 5-HT (1 μmol/L) plus fluoxetine (1 μmol/L), or 5-HT (1 μmol/L) plus fluvoxamine (1 μmol/L). Aorta were incubated with inhibitor for 30 minutes before 5-HT was added (15-minute incubation). We measured 5-HIAA and 5-HT concentration in aorta after incubation by using high-pressure liquid chromatography and normalized 5-HIAA and 5-HT concentration to aortic protein content (ng/mg protein; Figure 2). Aorta actively took up exogenous 5-HT, whereas both 5-HTT inhibitor fluvoxamine and fluoxetine reduced this 5-HT uptake in aorta from all of the animals studied (Figure 2).

To quantify total 5-HT uptake after incubation with exogenous 5-HT, we subtracted the 5-HT concentration in aorta incubated with vehicle from that in aorta incubated with exogenous 5-HT (5-HT 1 μmol/L—vehicle, [ng/mg protein], SHAM(D)=3.14±0.22, DOCA=2.36±0.31; P<0.05; SHAM(L)=3.50±0.39, LNNA=2.51±0.25; P<0.05; WKY=2.29±0.25 and SHR=2.08±0.40; Figure 2C, 2F, and 2H). These results suggest that aorta from DOCA and LNNA rats took up less 5-HT compared with their normotensive rats, whereas no difference existed between aorta from SHRs and WKYs. Neither the 5-HTT inhibitor fluoxetine (1 μmol/L) nor fluvoxamine (1 μmol/L) blocked the entire 5-HT uptake stimulated by exogenous 5-HT (Figure 2A, 2B, 2D, 2E, 2G, and 2H).

To quantify the 5-HTT–mediated 5-HT uptake in aorta, we compared 5-HT uptake in the presence and absence of fluvoxamine or fluoxetine. We subtracted the 5-HT concentration (nanograms per milligram of protein) in aorta after incubation with exogenous 5-HT plus 5-HTT inhibitor (fluvoxamine or fluoxetine) from that of aorta only incubated with exogenous 5-HT [5-HT−(5-HT+fluvoxamine) or 5-HT−(5-HT+fluoxetine)]. This is a value that we interpreted to reflect 5-HTT–dependent 5-HT uptake. There was significantly lower 5-HTT–mediated 5-HT uptake (calculated from fluvoxamine inhibition) in DOCA (1.83±0.12 ng/mg protein) compared with SHAM(D) (2.54±0.30 ng/mg protein) and in LNNA (1.45±0.20 ng/mg protein) compared with its normotensive control (2.18±0.23 ng/mg protein), whereas no difference existed in SHRs (1.05±0.32 ng/mg protein) compared with WKYs (1.23±0.22 ng/mg protein). Similar results were obtained from the calculation of 5-HTT–mediated 5-HT uptake by fluoxetine inhibition (ng/mg protein, SHAM(D)=1.92±0.12, DOCA=1.43±0.18; P=0.056; SHAM(L)=2.71±0.25, LNNA=1.91±0.20; P<0.05; and WKY=0.89±0.33, SHR=1.10±0.47).

Figure 1. A, SBPs of SHAM(D), DOCA, SHAM(L), LNNA, WKY, and SHR. B, Detection of basal concentrations of 5-HIAA and 5-HT in thoracic aorta from SHAM(D) rats, DOCA-salt hypertensive rats, SHAM(L) rats, LNNA hypertensive rats, SHRs, and WKYs. Bars, mean±SEM for the number of animals in parentheses. *Statistically significant differences (P<0.05) when compared with appropriate normotensive control. #Statistically significant differences (P<0.05) when compared with SHR.
Measurement of 5-HTT Expression in Aorta From Normotensive and Hypertensive Rats

Western blotting analysis using a 5-HTT antibody, which recognizes amino acids (516 to 630) mapping at the carboxy terminus of the 5-HTT protein (H115 antibody, Santa Cruz Biotechnology) showed a single band slightly lower than 75 kDa (Figure 3). The expression of 5-HTT in aorta from DOCA-salt rats was significantly elevated compared with SHAM(D) (arbitrary unit, SHAM(D)/H11005 = 2774 ± 469, DOCA/salt/H11005 = 4374 ± 125; P < 0.05), which is consistent with our previous report. Upregulation of 5-HTT protein expression was not caused by increased transcription but increased translation of mRNA; as we reported previously, we could not detect a difference in the ΔC_{T} value of 5-HTT in SHAM and DOCA-salt samples in a previous real-time RT-PCR study [ΔC_{T}(5-HTT C_{T} – GAPDH C_{T}) SHAM(D)=1.75 ± 0.1, DOCA-salt=2.45 ± 0.13; P=0.064; n=6]. Similarly, 5-HTT expression was also upregulated in LNNA rat aorta (arbitrary unit, SHAM(L)/H11005 = 2074 ± 291, LNNA=3603 ± 169; P<0.05). However, we did not observe a significant difference in 5-HTT aortic expression between

Figure 2. Effect of a 30-min preincubation with 5-HTT inhibitor fluoxetine (1 μmol/L) or fluvoxamine (1 μmol/L) on 5-HT concentration in aorta from pargyline-treated SHAM(D) rats (A), DOCA-salt hypertensive rats (B), SHAM(D) rats (D), LNNA-salt hypertensive rats (E), WKYs (G), and SHRs (H). Comparison of total 5-HT uptake and 5-HTT-dependent 5-HT uptake in aorta from SHAM(D) and DOCA-salt rats (C), SHAM(L) and LNNA hypertensive rats (F), WKYs and SHRs (I). Reported as 5-HT concentrations in nanograms per milligram of protein. Bars, mean±SEM for the number of animals in parentheses. *Statistically significant differences (P<0.05) when compared with vehicle. #Statistically significant differences (P<0.05) when compared with 5-HT (1 μmol).

Figure 3. Western blotting analysis of 5-HTT in homogenates from SHAM(D), DOCA-salt hypertensive rats, SHAM(L), LNNA hypertensive rats, WKY, and SHR aorta. Representative of 3 experiments/rats.
Discussion

The role of 5-HT and, more recently, its regulator 5-HTT in hypertension is controversial and intriguing. The enhancement of potency for 5-HT in inducing vascular contraction or pressor response in hypertension is striking. Increases in reactivity to 5-HT have been observed in a number of different forms of hypertension models, including DOCA-salt hypertensive rats, SHRs, and in human patients. Many mechanisms may contribute to this hyperresponsiveness, such as changes in 5-HT receptor signaling or by changing circulating levels of 5-HT. It makes logical sense that a change in the activity of the 5-HT regulator (5-HTT) would change the circulating 5-HT concentration and, thus, change the responsiveness of arteries to 5-HT.

In this study, we compared the 5-HTT function by testing 5-HT uptake of aorta from normotensive and hypertensive rats and tested whether the upregulation of 5-HTT expression and impaired function was common to hypertension by using 3 hypertension models, the DOCA-salt rats, LNNA rats, and SHRs.

Basal 5-HT Concentration Was Reduced in Aorta of DOCA-Salt and LNNA Hypertensive Rats But Not in SHRs

Free-circulating plasma 5-HT levels were reported as 15 to 120 nmol/L. 5-HT has relatively low affinity with the 5-HT2A receptor, which is the major receptor mediating 5-HT–induced arterial contraction in normotensive animals (inhibition constant, 100 to 3000 nmol/L). Theoretically, this freely circulating concentration of 5-HT cannot activate 5-HT2A receptors in a physiological situation, which supports the idea that the serotonergic system does not play a role in blood pressure regulation or hypertension. However, many reports showed that plasma 5-HT concentrations and arterial 5-HT receptors are changed in hypertension. In essential hypertensive humans and cyclosporine-induced hypertensive rats, reduced levels of platelet 5-HT and increased plasma 5-HT concentration were measured. Consistent with these reports, unpublished work from our laboratory determined that the DOCA-salt rat platelet 5-HT was reduced (ng/mL of whole blood, SHAM = 215.6 ± 35.4 and DOCA = 150.4 ± 36.7), whereas free-circulating 5-HT concentration was increased (ng/mL whole blood, SHAM = 5.82 ± 1.14 and DOCA = 28.1 ± 1.11). The current observation of reduced basal 5-HT content in aorta from DOCA-salt rats suggests that arteries, probably like platelets, function as a reservoir for 5-HT with decreased storage capacity and/or decreased 5-HT uptake ability in DOCA-salt hypertension. We speculate that the increased free-circulating 5-HT in DOCA-salt rats may result from decreased 5-HT storage in and uptake by both platelets and arteries. The increased extracellular free-circulating 5-HT in DOCA-salt hypertensive rats may have significant effects. 5-HT has high affinity for the 5-HT2B receptor (inhibition constant, 10 nmol/L), and the expression and function of 5-HT2B receptor is upregulated in arterial smooth muscle in DOCA-salt hypertensive rats. This receptor is activated endogenously to maintain the high blood pressure of DOCA rats. Moreover, nanomolar concentrations of 5-HT potentiate arterial contraction to endothelin and norepinephrine. Thus, it is possible that increased free-circulating 5-HT concentration induces vasoconstriction, increases total peripheral resistance, and, thus, elevates blood pressure in DOCA-salt hypertensive rats.

Unfortunately, there is no literature report about measurements of platelet 5-HT content or free-circulating 5-HT concentration in LNNA hypertensive rats. Our observation of decreased basal aortic 5-HT and 5-HIAA concentration suggests a changed 5-HTT activity and changed level of circulating 5-HT in LNNA rats. Similar to the DOCA-salt rat, 5-HT3B receptor expression and function is increased in LNNA rats and is necessary for maintained elevated blood pressure. It is possible that these changed factors of the serotonergic system in LNNA rats play roles in increasing and maintaining blood pressure. Further studies need to be done to investigate the change of platelet and free-circulating 5-HT in LNNA rats.

Only 2 studies in 1985 investigated platelet 5-HT level in SHRs. As opposed to a decreased 5-HT in platelets in DOCA-salt hypertensive rats, SHRs have more platelets and similar platelet 5-HT levels in SHRs compared with WKYs. Free-circulating 5-HT in SHRs has not been reported. Our observation of similar basal 5-HT levels in aorta from SHRs and WKYs is consistent with these reports in platelets. We are aware that the basal level of 5-HT concentration is lower in WKYs compared with Sprague–Dawley normotensive rats. This may suggest an inherent difference in these strains. The serotonergic system may have a different degree of impact on blood pressure regulation in WKYs compared with Sprague–Dawley rats.

It is important to understand arterial function of 5-HTT in hypertension not only as a reflection of circulating 5-HT levels but also because intracellular 5-HT may have a function. Intracellular 5-HT was found to be used to transamidate (covalently modify) small GTPases by transglutaminases, rendering GTPases such as RhoA constitutively active. There are other intracellular proteins important for smooth muscle contraction reported as substrates of transglutaminase, such as actin, myosin, and troponin. It is possible that arterial smooth muscle cell intracellular 5-HT concentration regulates contraction by changing protein activity. Changing intracellular 5-HT concentration would, in turn, change arterial contraction. Thus, our observation of an altered basal activity in DOCA-salt and LNNA hypertension may have consequent pathologic results.

5-HT Uptake Ability Was Reduced in Aorta of DOCA-Salt and LNNA Hypertensive Rats But Not in SHRs

By normalizing 5-HT content to protein concentration and comparing the change of 5-HT concentration in nanograms per milligram of protein, our results showed a decreased total 5-HT uptake and a decreased 5-HT–dependent 5-HT uptake (Figure 2C and 2F) in aorta from DOCA-salt and LNNA hypertensive rats exposed to exogenous 5-HT compared with that of aorta from their normotensive SHAM rats. We did not observe differences in 5-HT uptake in aorta from SHRs and WKYs. Our results suggest that 5-HTT function was impaired in DOCA-salt and LNNA hypertensive rats. The
decreased total 5-HT uptake observed in DOCA-salt and LNNA hypertensive rats may account for decreased basal aortic 5-HT content in both models and increased free-circulating 5-HT (DOCA-salt hypertension). Possible explanations for the contradictory findings of an upregulated 5-HTT protein expression and a decreased 5-HTT function in DOCA-salt and LNNA rats includes different membrane/cytosol distribution of 5-HTT in these SHAM and hypertensive animals, as well as translational modification of 5-HTT protein. Phosphorylation of 5-HTT by p38 mitogen-activated protein kinase increases 5-HTT function by stimulating insertion of intracellular 5-HTT into the cell membrane and increasing total 5-HTT activity. By contrast, protein kinase C reduces 5-HTT function by phosphorylating 5-HTT protein and causing translocation of 5-HTT to the cytosol. Thus, it is possible that total expression of 5-HTT increased in DOCA and LNNA hypertensive animals, whereas the membrane fraction or functional 5-HTT actually decreased.

We did not observe a difference in basal aortic 5-HT content, 5-HT uptake ability, or 5-HTT protein expression in aorta from SHR compared with WKY rats. There are several factors that may contribute to the differences that we observed. First, as we discussed above, the strain is different (WKY versus Sprague–Dawley). Second, the SBPs of SHRs (172 ± 7 mm Hg) were lower than DOCA-salt rats (197 ± 6 mm Hg) and LNNA rats (228 ± 9 mm Hg). The change of 5-HTT expression and function may only happen in severe hypertension. Third, it is possible that the DOCA that we used in our DOCA-salt hypertension model causes the different 5-HTT expression and function. However, Kulikov et al. reported that stimulation of mineralocorticoid receptors had no effect on 5-HTT radioligand binding density in rat midbrain. Furthermore, the observation of impaired 5-HTT function was also obtained in LNNA hypertensive rats. Thus, it is unlikely that the change of 5-HTT function in DOCA-salt hypertension is from effects of mineralocorticoid.

More importantly, the correlation between 5-HTT receptor and plasma 5-HT or 5-HTT function was reported recently. Callebert et al. observed a significantly increased plasma serotonin levels in wild-type mice with upregulated 5-HTT receptor after exposure to chronic hypoxia but not in 5-HTT receptor−/− mice. Acute treatment with 5-HT receptor agonist induced a rapid 5-HTT and 5-HT receptor–dependent increase of plasma serotonin levels, which suggests that 5-HTT receptor activation inhibits 5-HTT function and, thus, less 5-HT being taken up. Upregulated 5-HT receptor expression and function in DOCA and LNNA hypertension have been reported. On the other hand, the 5-HT receptor was not involved in 5-HT–induced vasoconstriction in the hindquarters of SHRs, which suggests that 5-HT receptor was not activated in SHRs as it was in DOCA-salt and LNNA hypertensive rats. Our results are consistent with the report by Callebert et al. in that we observed an increased free-circulating 5-HT with increased 5-HT receptor function while decreased 5-HTT function in DOCA rats, increased 5-HT receptor function while decreased 5-HT function in LNNA rats, no change of 5-HT receptor, and normal 5-HTT activity in SHR. How the 5-HT receptor regulates 5-HTT is not yet clear.

**Other Arterial 5-HT–Uptake Mechanisms**

One interesting observation in this study was that a maximal concentration of fluoxetine (1 μmol/L) and fluvoxamine (1 μmol/L) did not block the entire uptake stimulated by exogenous 5-HT. This suggests that there are other mechanisms by which 5-HT is transported into aorta. The norepinephrine transporter, dopamine transporter, organic cation transporter 1, and organic cation transporter 3 are possible candidates responsible for 5-HT uptake.

It is important to note that we studied 1 concentration of exogenous 5-HT at 1 time point in our active uptake study. Studies that examine 5-HT uptake at various times and with a range of 5-HT concentrations need to be done to compare 5-HTT Michaelis constant and maximum velocity values in aorta from DOCA-salt, LNNA hypertensive rats, and their SHAM control rats. However, this is difficult without determination of other mechanisms for 5-HT uptake. Thus, kinetic studies have to wait until these alternative mechanisms are discovered.

**Perspectives**

We demonstrated a change in the basal level of arterial 5-HT concentration and 5-HTT function in aorta from DOCA-salt and LNNA hypertensive rats but not SHR compared with appropriate control. By actively taking up 5-HT, peripheral arteries may play a role in decreasing circulating 5-HT concentration. We speculate that peripheral arteries actively take up 5-HT as a protective mechanism of the body to keep a normal level of free-circulating 5-HT and to prevent blood pressure increases. Furthermore, our findings of lower basal levels of 5-HT and impaired 5-HT–uptake function of 5-HTT in DOCA-salt and LNNA rats suggest that losing the arterial 5-HT clearance mechanism in these animals might lead to elevated blood pressure. Investigation of the altered arterial function in hypertensive animals would help us understand the pathogenesis of hypertension.

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**Disclosures**

None.

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